Anthraquinones from the Roots of *Prismatomeris tetrandra*

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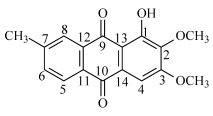
Three new anthraquinones, 1-hydroxy-2,3-dimethoxy-7-methyl-9,10-anthraquinone, 1,3-dihydroxy-5,6-dimethoxy-2-methyl-9,10-anthraquinone, and 3-hydroxy-1,5,6-trimethoxy-2-methyl-9,10-anthraquinone, along with five known anthraquinones were isolated from the roots of *Prismatomeris tetrandra*. Their structures were determined on the basis of spectroscopic data.

Key words Rubiaceae; *Prismatomeris tetrandra*; anthraquinone; 1-hydroxy-2,3-dimethoxy-7-methyl-9,10-anthraquinone; 1,3-dihydroxy-5,6-dimethoxy-2-methyl-9,10-anthraquinone; 3-hydroxy-1,5,6-trimethoxy-2-methyl-9,10-anthraquinone

The genus *Prismatomeris* (Rubiaceae) comprises 25 species in the world, of which approximately grow in tropical Asia, while only *Prismatomeris labordei* (LEVL.) MERR. and *Prismatomeris tetrandra* (ROXB.) K. SCHUM are found in China.[1] *P. tetrandra* is a small shrub distributed in southern regions of China.[1] The roots of the plant were used as Chinese traditional medicine to treat leucocythemia, gum bleeding, hepatitis, and anaemia.[2] An extract of *P. tetrandra* was found to be cytotoxic in brine shrimp lethality assay and on human tumor cell line,[3] however, few chemical constituents from this plant were reported so far, except organic aluminium salt, tectoquinone, rubiadin, rubiadin-1-methyl ether, damnacanthal, β-sitosterol,[4] ursolic acid, and daucosterol.[3] In the course of our continuing search for the bioactive constituents of the plant, three new anthraquinones, 1-hydroxy-2,3-dimethoxy-7-methyl-9,10-anthraquinone (**1**), 1,3-dihydroxy-5,6-dimethoxy-2-methyl-9,10-anthraquinone (**2**), and 3-hydroxy-1,5,6-trimethoxy-2-methyl-9,10-anthraquinone (**3**), were isolated together with five known compounds, namely, 1-hydroxy-2-methyl-9,10-anthraquinone (**4**),[5] 1,3-dihydroxy-2-methoxy-9,10-anthraquinone (**5**),[5] 1,3-dihydroxy-2-methyl-9,10-anthraquinone (rubiadin **6**),[6] 3-hydroxy-1-methoxy-2-methyl-9,10-anthraquinone (rubiadin-1-methyl ether **7**),[6] 2-hydroxy-3-hydroxymethyl-9,10-anthraquinone (**8**),[5] from the roots of this plant. Their structures were elucidated by the spectroscopic means.



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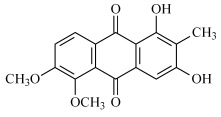

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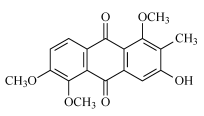

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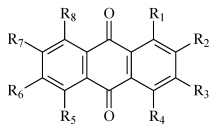

4 R_1=OH R_2=CH_3 R_3= R_4= R_5= R_6 =R_7= R_8=H

5 R_1=R_3=OH R_2=OCH_3 R_4= R_5= R_6 =R_7= R_8=H

6 R_1=R_3=OH R_2=CH_3 R_4= R_5= R_6 =R_7= R_8=H

7 R_1=OCH_3 R_2=CH_3 R_3=OH R_4= R_5= R_6 =R_7= R_8=H

8 R_1= R_4= R_5= R_6 =R_7= R_8=H R_2=OH R_3=CH_2OH

Compound **1** was obtained as red needle crystals. The formula was determined to be $C_{17}H_{14}O_5$ on the basis of high resolution (HR) EI-MS, which gave an ion peak at *m*/*z* 298.0845 $[M]^+$ (Calcd 298.0841). The IR spectrum of **1** showed the presence of hydroxyl groups (3438 cm^{-1}), conjugated carbonyl (1668, 1640 cm^{-1}) and aromatic rings (1601, 1504 cm^{-1}). In the UV spectrum of **1**, it gave the absorption at 220, 281, and 358 nm, suggesting the existence of the skeleton of anthraquinone. The ^{13}C-NMR spectrum of **1** (Table 1) showed 17 carbon signals. Except for 14 carbon signals of the anthraquinone skeleton, one methyl carbon signal, and two methoxy carbon signals were observed. The ^{1}H-NMR spectrum of **1** (Table 1) showed four aromatic proton signals, an ABX system at δ_H 8.16 (1H, d, J=8.0 Hz, H-5), 7.58 (1H, dd J=8.0, 2.0 Hz, H-6), 8.09 (1H, d J=2.0 Hz, H-8), and a singlet signal at δ_H 7.48 (1H, s). In addition, a methyl signal at δ_H 2.54 (3H, s), two methoxy signals at δ_H 4.02 (3H, s) and 4.06 (3H, s), and an aromatic hydroxy signal at δ_H 12.83 (1H, s) were presented in the ^{1}H-NMR spectrum. Thus, an anthraquinone with four substitutions can be deduced, in which one of the rings of the anthraquinone was mono-substitution, and another ring was tri-substitution. Furthermore, the positions of these groups were given by the detailed heteronuclear multiple bond connectivity (HMBC) analysis. In the HMBC spectrum (Fig. 1), a correlation between the single aromatic proton at δ_H 7.48 (1H, s) and carbonyl carbon at δ_C 181.8 (C-10) was presented, suggesting the proton was located at C-4. While long-range correlations of aromatic hydrogen at δ_H 7.58 (1H, dd J=8.0, 2.0 Hz, H-6) and 8.09 (1H, d, J=2.0 Hz, H-8) with the methyl carbon at δ_C 21.9 and the aromatic hydrogen at δ_H 8.09 (1H, d, J=2.0 Hz) with the carbonyl carbon at δ_C 188.0 (C-9) were indicative of that the methyl was at C-7. In addition, such downfield aromatic hydroxy proton at δ_H 12.83 (1H, s) and long-range correlations of this proton with C-13 at δ_C 112.4 and C-2 at δ_C 141.4 were suggestive of that the hydroxy must be located at C-1. Combined all the information above, the structure of **1** is identified as 1-hydroxy-2,3-dimethoxy-7-methyl-9,10-anthraquinone.

Compound **2** was obtained as yellow powder. It showed absorptions at 222, 278, and 361 nm in the UV spectrum, implying the presence of the skeleton of anthraquinone. The HR-EI-MS gave the formula of $C_{17}H_{14}O_6$, which gave an ion

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1**—**3**

Position	**1**[a]		**2**[b]		**3**[b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		156.6		162.7		159.9
2		141.4		116.5		125.3
3		158.3		161.9		161.3
4	7.48 (1H, s)	104.0	7.18 (1H, s)	107.0	7.40 (1H, s)	108.7
5	8.16 (1H, d, J=8.0 Hz)	127.4		149.1		148.0
6	7.58 (1H, dd, J=8.0, 2.0 Hz)	135.1		158.8		157.5
7		145.3	7.53 (1H, d, J=8.5 Hz)	117.0	7.50 (1H, d, J=8.5 Hz)	117.2
8	8.09 (1H, d, J=2.0 Hz)	127.1	8.03 (1H, d, J=8.5 Hz)	124.4	7.94 (1H, d, J=8.5 Hz)	124.2
9		188.0		185.4		179.4
10		181.8		181.1		181.9
11		131.2		126.1		125.6
12		133.3		126.2		128.1
13		112.4		108.3		117.4
14		129.3		133.4		135.5
Me-2			2.06 (3H, s)	8.0	2.12 (3H, s)	8.9
Me-7	2.54 (3H, s)	21.9				
MeO-1					3.75 (3H, s)	60.6
MeO-2	4.02 (3H, s)	61.0				
MeO-3	4.06 (3H, s)	56.5				
MeO-5			3.79 (3H, s)	60.5	3.79 (3H, s)	60.5
MeO-6			3.95 (3H, s)	56.4	3.92 (3H, s)	56.3
OH-1	12.83 (1H, s)		13.22 (1H, s)			
OH-3			11.14 (1H, s)		11.00 (1H, s)	

a) Measured in $CDCl_3$, *b*) measured in DMSO-d_6.

peak at *m*/*z* 314.0775 $[M]^+$ (Calcd 314.0790). The ^{1}H-NMR spectrum of **2** (Table 1) showed the presence of two aromatic *ortho*-coupled protons at δ_H 8.03 (1H, d, J=8.5 Hz, H-8) and 7.53 (1H, d, J=8.5 Hz, H-7), a singlet aromatic proton at δ_H 7.18 (1H, s), two phenolic hydroxyl group at δ_H 11.14 (1H, s) and 13.22 (1H, s), a methyl group at δ_H 2.06 (3H, s), and two methoxy groups at δ_H 3.79 (3H, s) and 3.95 (3H, s). Combined the ^{13}C-NMR information of **2** (Table 1), in which 17 carbon signals were shown including a methyl carbon at δ_C 8.0 and two methoxy carbon signals at δ_C 56.4 and δ_C 60.5, an anthraquinone with five substitutes were deduced. The exact substitute positions were also confirmed by HMBC experiment (Fig. 2). In the HMBC experiment, the correlations were given between the methyl proton signal at δ_H 2.06 (3H, s) and three aromatic carbons at δ_C 162.7 (C-1), 161.9 (C-3), and 116.5 (C-2), indicating the methyl groups was at C-2. In addition, the correlations were shown between the single aromatic proton signal at δ_H 7.18 and carbon signals at δ_C 181.1 (C-10), 116.5 (C-2), and 108.3 (C-13), between the proton signal at δ_H 8.03 (1H, d, J=8.5 Hz, H-8) and carbon signals at (δ_C 185.4 (C-9), 158.8 (C-6), and 126.1 (C-11), suggesting the single proton located at C-4, and the two aromatic *ortho*-coupled protons located at C-7 and C-8, respectively. These correlations together with the above data support the structure **2** of a 1,3-dihydroxy-5,6-dimethoxy-2-methyl-9,10-anthraquinone.

Compound **3** was obtained as yellow needle crystals, mp 256—258 °C, The UV spectrum showed the absorptions at 219, 275, and 346 nm, and the IR spectrum showed bands at 3329 and 1666 cm^{-1} due to hydroxyl and carbonyl groups. These data suggested that **3** was also an anthraquinone. The HR-EI-MS gave the formula of $C_{18}H_{16}O_6$, which gave an ion peak at *m*/*z* 328.0952 $[M]^+$ (Calcd 328.0947). The ^{1}H-NMR (Table 1) spectrum of **3** demonstrated the two aromatic

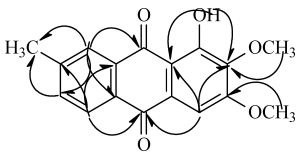

Fig. 1. Selected HMBC Correlations for **1**

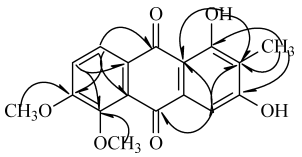

Fig. 2. Selected HMBC Correlations for **2**

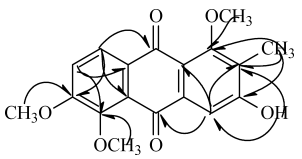

Fig. 3. Selected HMBC Correlations for **3**

ortho-coupled protons, three methoxy, one methyl, a single aromatic proton, and one hydroxy. The ^{13}C-NMR (Table 1) gave 18 carbon signals. Comparing with the corresponding data of **2** (Table 1), the ^{1}H- and ^{13}C-NMR data of **3** were similar to those of **2** except a methoxy in **3** instead of a hydroxy in **2**. The position of the methoxy group was located at C-1 on the basis of HMBC analysis (Fig. 3). This was further confirmed from the fact that the downfield hydroxy proton signal at δ_H 13.22 (1H, s) in **2** disappeared and carbon signals of C-1, C-9 and C-13 in **3** upfield shifted by 3, 6, and 9 ppm, respectively. All substitute groups were attribut-

ed by heteronuclear multiple quantum coherence (HMQC) and HMBC (Fig. 3) experiment. So, the structure of **3** was identified as 3-hydroxy-1,5,6-trimethoxy-2-methyl-9,10-anthraquinone.

Experimental

General Experimental Procedures Melting points were determined on an XT-4 micro-melting point apparatus and are uncorrected. UV spectra were obtained on a Shimadzu UV-240 instrument. IR spectra were recorded on an IMPACT 400 (KBr) spectrometer. ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), HMQC and HMBC spectra were run on an INOVA-500 spectrometers with TMS as internal standard and values were given in ppm (δ). EI-MS and HR-EI-MS were performed on Autospec Ultima-TOF mass spectrometer. Electrospray ionization (ESI) was performed on Agilent 1100 series LC/MSD Trap mass spectrometer (SL). Silica gel (100—200, 200—300 mesh) (Qingdao) was used for CC and silica gel GF-254 (Qingdao) for TLC and preparative TLC.

Plant Material The roots of *Prismatomeris tetrandra* (Roxb.) K. Schum. were collected in Hainan Province, China, in Aug. 2003, and were identified by Prof. ShiMan Huang, Hainan University. A voucher specimen is deposited in the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences.

Extraction and Isolation The dried, milled roots (6.0 kg) were extracted with 95% EtOH to afford residue (326 g) on removal of solvent under reduced pressure. The EtOH extract was suspended in H_2O and then partitioned in turn using petroleum ether, EtOAc, and *n*-BuOH, the EtOAc-soluble fraction was evaporated under a vacuum to give an residue (56 g), which was chromatographed over silica gel (200—300 mesh) and eluted with solvents of increasing polarity (petroleum ether–acetone) to give 15 fractions, Fr. 5 was crystallized in acetone to give compound **5** (60 mg). Fr. 6 was crystallized in acetone to give compound **6** (82 mg). Fr. 7 was crystallized in acetone to give compound **7** (1000 mg). The fractions from 1—2 were combined, then chromatographed over silica gel (200—300 mesh) and eluted with different solvents of increasing polarity (petroleum ether–EtOAc) to yield compound **4** (10 mg), which was crystallized in acetone. Similarly, Fr. 3 was further chromatographed using petroleum ether–EtOAc to yield compound **1** (12 mg). Combined fraction (Fr. 8—Fr. 12) was further chromatographed using petroleum ether–acetone to give compound **2** (8 mg), **3** (11 mg), and **8** (10 mg).

8-Hydroxy-6,7-dimethoxy-2-methyl-9,10-anthraquinone (**1**): Red needles (acetone), mp 159—161 °C, UV λ_{max} (MeOH) nm: 220, 281, 358. IR (KBr) (cm^{-1}): 3438, 2941, 1668, 1640, 1601, 1504, 1446, 1427, 1365, 1311, 1275, 1238, 1146, 1107, 1049, 985, 864, 762. EI-MS *m*/*z*: 298, 283, 269, 255, 184, 149. ESI-MS, *m*/*z*: 321 $[M+Na]^+$, 619 $[2M+Na]^+$, HR-EI-MS, *m*/*z*: 298.0845 $[M]^+$ (Calcd for $C_{17}H_{14}O_5$: 298.0841). ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data see Table 1.

1,3-Dihydroxy-5,6-dimethoxy-2-methyl-9,10-anthraquinone (**2**): Yellow powder (acetone), mp >228 °C dec. UV λ_{max} (MeOH) nm: 222, 278, 308, 361. IR (KBr) (cm^{-1}): 3342, 1670, 1618, 1568, 1483, 1435, 1381, 1344, 1271, 1049, 806. EI-MS *m*/*z*: 314, 298, 285, 281, 280, 269, 267, 255. HR-EI-MS, *m*/*z*: 314.0775 $[M]^+$ (Calcd for $C_{17}H_{14}O_6$: 314.0790). ^{1}H-NMR (DMSO-d_6, 500 MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz) data see Table 1.

3-Hydroxy-1,5,6-trimethoxy-2-methyl-9,10-anthraquinone (**3**): Yellow needles (acetone), mp 256—258 °C, UV λ_{max} (MeOH) nm: 193, 219, 275, 297, 346, 383. IR (KBr) (cm^{-1}): 3329, 1666, 1572, 1481, 1338, 1265, 1130, 1053, 991, 947, 843, 781, 723. EI-MS *m*/*z*: 328, 314, 313, 299, 297, 295, 269, 253, 227. HR-EI-MS, *m*/*z*: 328.0952 $[M]^+$ (Calcd for $C_{18}H_{16}O_6$: 328.0947). ^{1}H-NMR (DMSO-d_6, 500 MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz) data see Table 1.

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